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**Establishing Natural Product Content with the Natural Radiocarbon
Signature**

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Radiocarbon (^{14}C) is produced naturally at relatively constant levels in the atmosphere by cosmic ray interactions with nitrogen. Isolated carbon atoms are quickly oxidized to CO_2 in the atmosphere and are incorporated into biomolecules. Above ground nuclear weapons testing between 1955-63 caused a pulse in the ^{14}C content of the atmosphere over the past 55 years, but it is now almost back to the 1950 level. All living things are labeled with ^{14}C while alive and retain the isotopic signature at death and when processed into food. Carbon from petroleum sources is devoid of ^{14}C and easily distinguished from natural biological sources ($^{14}\text{C}/\text{C} = 1.2$ parts per trillion) by accelerator mass spectrometry (AMS). We will briefly explain how AMS works and present examples of how it is used to determine natural and fossil carbon content of food. We will also describe how AMS can be used to verify if a claimed vintage is consistent with its isotopic signature.

A natural product is generally thought to be derived from a plant or animal that was recently living. It may have undergone some chemical processing, but we usually view that processing as separatory, isolating specific compounds for use as a flavor, additive or possibly a preservative in a processed food. On the other end of the spectrum are artificial compounds synthesized from petroleum derived precursors. These compounds may occur in nature, but large scale synthesis can be much less expensive than purification from a natural source

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where the desired compound could be in low abundance or difficult to isolate from a complex mixture. Between the extremes of purely bio-derived and petroleum-derived compounds are chemically manipulated natural products. In these a natural product is manipulated to change functional groups or saturate bonds, for example. These products are often low-calorie or poorly absorbed compounds that retain a flavor of a natural product while reducing sugar or fat content. Many processed foods are mixtures of these three types of ingredients.

There are many processed foods that claim to be “all natural” which implies that they consist solely of bio-derived ingredients. All-natural products can often demand a higher price as consumers deem them of superior quality. How can an all-natural product be distinguished from petroleum-derived one if they are chemically identical? Bio-derived carbon and petroleum-derived carbon possess distinct isotopic signatures that can be used to make this distinction.

Radiocarbon analysis is traditionally utilized as a tool for archeological dating (1) rather than natural product authentication. Radiocarbon or carbon-14 (^{14}C) is produced naturally in the atmosphere by cosmic ray interactions with nitrogen-14. Single carbon atoms in the atmosphere are chemically reactive and are quickly oxidized to carbon dioxide (CO_2). The CO_2 from the atmosphere is incorporated into plants and works its way up the food chain to label every living thing with ^{14}C . All living things reflect the isotopic signatures of their food sources. The natural atmospheric concentration of $^{14}\text{C}/\text{C}$ has remained relatively stable at about 1.2 parts per trillion over the past three thousand years (2). Anthropogenic activities over the past century are shifting the atmospheric $^{14}\text{C}/\text{C}$ concentration. Burning fossil fuels increased the concentration of CO_2 in the atmosphere with ^{14}C -free carbon. ^{14}C has a radioactive half-life of 5730 years, so fossil fuel derived carbon that has been dead for millions of years is ^{14}C -free. The slow decline in atmospheric $^{14}\text{C}/\text{C}$ observed in the first half of the 20th century is known as the Suess Effect (3).

Atmospheric testing of nuclear weapons during the 1950s and early 1960s almost doubled the concentration of $^{14}\text{C}/\text{C}$ in the atmosphere (Figure 1) (4,5). From the peak in 1963, the level of $^{14}\text{CO}_2$ has decreased with a mean life of about 16 years, not due to radioactive decay, but due to mixing with large marine and terrestrial carbon reservoirs. The ^{14}C has not actually disappeared, it has simply moved out of the atmosphere. In recent years the decline of atmospheric $^{14}\text{C}/\text{C}$ is believed to be driven more by CO_2 addition from combustion of fossil fuels than sequestration of elevated $^{14}\text{CO}_2$ into carbon reservoirs. The temporal variations of artificially high levels of atmospheric radiocarbon have been captured in organic material world-wide and provide a means to determine a date of synthesis for biomolecules. Since radiocarbon is

incorporated into all living things, this pulse is an isotopic chronometer of the past 55 years.

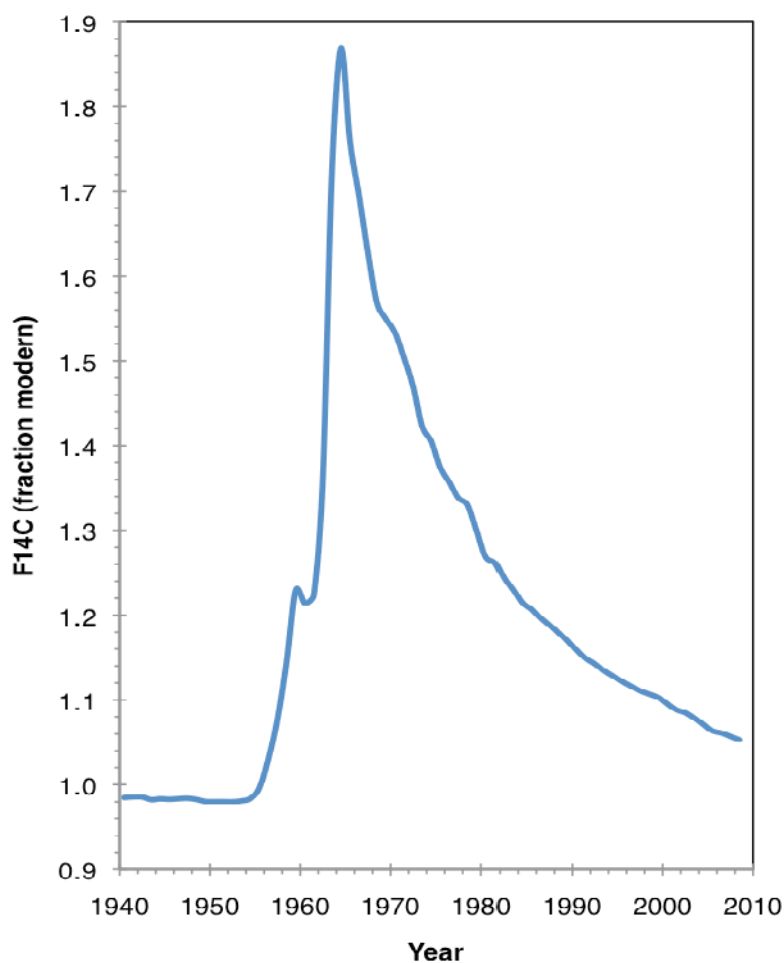


Figure 1. Northern hemisphere growing season average of atmospheric $^{14}\text{C}/\text{C}$ concentration in CO_2 from 1940-2008. The ^{14}C concentration curve is a compilation of extensive tree ring and atmospheric records (6-10). The F^{14}C (i.e., fraction modern) nomenclature is designed for expressing bomb-pulse ^{14}C concentrations (11).

The atmospheric $^{14}\text{CO}_2$ curve depicted in Figure 1 is a Northern Hemisphere annual growing season average. It is constructed using several independent data sets that used tree rings, recent plant growth, and direct atmospheric sampling to provide carbon samples (6-10). Since there were relatively few geographic sources of bomb-pulse ^{14}C , the upswing and the peak values of the curve do vary with geography (8,9,12). However, since CO_2 is a gas and did not fall out of the atmosphere like particulates, the pulse of $^{14}\text{CO}_2$ mixed in the atmosphere with all other CO_2 to produce a relatively homogeneous distribution of atmospheric $^{14}\text{CO}_2$ by about 1970 (9).

The isotopic content of new plant growth reflects the atmospheric $^{14}\text{C}/\text{C}$ concentration. Isotope fractionation effects observed in $^{13}\text{C}/\text{C}$ with C3 and C4 plants are also seen in $^{14}\text{C}/\text{C}$, so these corrections need to be included. About 1.1% of carbon is the stable isotope ^{13}C . Plants, to a variable degree, can discriminate between ^{12}C and ^{13}C , resulting in differences in the levels of this isotope between different types of plants (13,14). Furthermore, microclimate variations and environmental stresses can shift ^{13}C fractionation in a particular plant (14). Differences in the fixation of CO_2 during photosynthesis distinguish the more common C3 plants from C4 plants. C4 plants have a double fixation step for CO_2 and their photosynthetic pathway is located deeper in the leaves. Isotope fractionation in C4 plants is primarily limited by diffusion (13,14). This is in contrast to C3 plants which can better discriminate between these isotopes and both reduce the binding of ^{13}C and more readily make ^{13}C diffuse out through the stomatal pores to the outer atmosphere (13). As a result, C4 plants (which include corn and sugar cane) contain higher amounts of ^{13}C than C3 plants (which include potato, sugar beet, and wheat) (15). In general, C4 plants tend to grow in hotter or drier climates than C3 plants whose open stomata lose too much water to thrive. New leaves are produced in a matter of weeks while larger fruits and vegetables form over the period of a month or two. Herbivores lag the atmosphere slightly because their primary carbon source is on the order of weeks to months old. Omnivores and carnivores lag the atmosphere further because their carbon sources are further removed.

Within organisms, molecules turn over at different rates so ^{14}C levels can vary between molecules. For example, cellulose is relatively static, DNA incorporates new carbon at cell division, lipids can be stored for extended periods, and most other molecules cycle carbon rapidly. The date of formation of a tissue or specific biomolecule can be estimated from the bomb-curve by considering these lags in incorporation and relating the $^{14}\text{C}/\text{C}$ concentration with the date. The actual data record is relatively noisy because it consists of biweekly atmospheric grab samples (8-10). Most biomolecules in foods and additives are naturally produced over a growing season. Constructing a bomb-pulse curve from annual averages of the carbon intake over a growing season

smooths the curve and can account for much food chain lag. Caution must be exercised when relating an elevated $^{14}\text{C}/\text{C}$ measurement to a date of formation because the pulse is double valued. Placing a sample on the ascending or descending side of the pulse can often be accomplished if other information is available, e.g. tannins in red wine are a year older than the ethanol (12).

Today nearly all ^{14}C forensic analyses are conducted using accelerator mass spectrometry (AMS). AMS is much faster and generally more precise than decay counting since it separates carbon atoms by mass and counts individual ^{14}C atoms vs. a stable ^{13}C or ^{12}C current and is not constrained to wait for atomic decay. If a sample is large (~1 g carbon), readily soluble in liquid scintillation cocktail and does not cause quenching, scintillation counting can distinguish between biological and petroleum carbon sources, but may be challenged to distinguish relatively small depressions seen with a petroleum-derived additive in a largely natural sample. AMS uses smaller samples than decay counting (~1 mg carbon), an important issue when analyzing specific ingredients or compound classes in a sample.

Sample preparation and measurement details vary among AMS facilities, depending on the type of sample to be analyzed and the design of spectrometer. Routine radiocarbon analyses by AMS require about 1 mg carbon. Samples are dried completely and then combusted with excess oxygen to produce CO_2 . The CO_2 is purified to remove water vapor, nitrogen, oxides of nitrogen, and oxides of sulphur. It is then reduced to graphite or elemental carbon on metal catalyst, often cobalt or iron powder.

Primary standards, secondary standards, backgrounds, and samples under analysis are similarly processed to produce graphite, which is the form of carbon analyzed by the majority of AMS systems. Graphite is the preferred form of carbon because it can be made easily at high purity, produces intense negative ion currents, has very low vapor pressure (which produces minimal sample memory and allows fast switching of samples), and can be prepared at satellite labs and shipped to AMS facilities for analysis. A handful of gas accepting ion sources that take direct feed of CO_2 exist, but they are not widely used for high precision dating. Work is currently underway to develop an ion source interface that could accept a HPLC eluent and directly oxidize it to CO_2 for direct injection into a spectrometer. It is important to have consistent sample source material (e.g., all carbon graphite or CO_2) because different molecules ionize with different efficiencies. Using a single sample matrix for analyses eliminates the need to normalize for variable ionization efficiencies. Methods for producing graphite that were designed for elevated biological tracing experiments (16,17) can be used for natural product evaluation if exceptional care is taken to carefully determine backgrounds and the associated uncertainties (18).

Materials and Methods

Samples

All samples analyzed for natural carbon content were purchased in San Francisco Bay area grocery stores between 2007 and 2010. Products were selected to include a variety of processed foods containing natural and artificial sweeteners and flavorings. The foods could be separated into three general groups: baked goods, cereals, sauces and soft drinks consisting primarily of grains, sugars, and oils; powders requiring water for reconstitution; and specific flavorings.

Sample preparation

All samples were prepared in the Natural Radiocarbon Prep Lab at Lawrence Livermore National Laboratory (LLNL). Sample prep varied with sample type due to liquid content. Since AMS measures milligram-sized samples, excess water needs to be removed prior to combustion to CO₂. If water is not removed, it becomes water vapor during heating, creates high pressure and causes combustion tubes to explode in the furnace.

Dry powders and bakery products were weighed (2-5 mg) and added directly to combustion tubes. Liquid samples were added to combustion tubes and then dried over 2 days in a convection oven set at 95°C. Ethanol in the flavorings evaporated quickly but water in soft drinks took longer to evaporate. Excess copper oxide (CuO) was added to each dry sample, and the tubes were brought to vacuum and sealed with a H₂/O₂ torch. Tubes were placed in a furnace set at 900°C for 3.5 h to combust all carbon to CO₂. The evolved CO₂ was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors (19,20). Graphite samples were pressed into aluminum sample holder cathodes and carried to the spectrometer for analysis.

AMS Analysis

All ¹⁴C/C measurements were completed with graphite targets analyzed at the Center for Accelerator Mass Spectrometry at LLNL on the HVEE FN-class tandem electrostatic AMS system (Figure 2). The operation was similar to that

when performing high-precision measurements of dental enamel (21). The system employs a LLNL designed high-output negative ion solid graphite Cs-sputter source (22) which emits 250-350 μA of $^{12}\text{C}^-$ from a full-sized sample, corresponding to approx. 1000 ^{14}C counts per second from a contemporary sample. The FN AMS system routinely achieves 15 % total system efficiency for C analyzing $^{14}\text{C}^{4+}$ in the detector (23). Details on the design of the LLNL AMS system and its operation can be found in the literature (22-25). Natural product investigation samples are usually full sized and contemporary, so analysis times are relatively rapid, generally less than 5 minutes. Samples were measured for 30,000 ^{14}C counts per cycle for 4-7 cycle repetitions and achieved standard deviations of 0.2-1.5%.



Figure 2. HVEE-FN AMS system at LLNL.

A $\delta^{13}\text{C}$ fractionation correction of -25 ± 2 or -15 ± 2 was used for all samples based on the dominant carbon source for each sample (C3 or C4 plant). Corrections for background contamination introduced during sample preparation were made following standard procedures (26). All data were normalized with six identically prepared NIST SRM 4990B (Oxalic Acid I) standards. Isotopic

secondary standards NIST SRM 4990C (Oxalic Acid II), IAEA C-6 (ANU sucrose), and TIRI wood B (27) were used as quality control samples to monitor spectrometer performance. ^{14}C -free coal served as background material for processing the samples. Samples were organized in groups of 12 or 14 unknowns bracketed by primary standards with one primary standard in the middle of the group. The secondary standards, primary standards and group of unknowns are measured consecutively as a cycle. The set of standards and unknown samples are measured repeatedly until desired precision is achieved. A typical group of 14 natural product samples was measured completely in 2-3 h. All ^{14}C data are reported using the $F^{14}\text{C}$ fraction modern nomenclature developed for post-bomb data (11). $F^{14}\text{C}$ is a concentration unit ($^{14}\text{C}/\text{C}$) denoting enrichment or depletion of ^{14}C relative to oxalic acid standard normalized for isotope fractionation. $F^{14}\text{C}=1$ modern can be thought of as the natural atmospheric concentration of $^{14}\text{C}/\text{C}$ before anthropogenic activities started to influence the ratio. It is actually derived from multi-decadal average of $^{14}\text{C}/\text{C}$ from pre- and early industrial revolution tree rings from the 19th century (28).

Results and Discussion

The natural products purchased between 2007 and 2010 should contain carbon from 2005 to 2009 growing seasons. Terrestrial natural products in this survey should possess $F^{14}\text{C} = 1.03$ -1.07. The results of the measurements and assessment of the origin of the carbon are listed in Tables 1-3.

Table 1 contains $^{14}\text{C}/\text{C}$ results for baked goods, cereals, sauces and soft drinks consisting primarily of grains, sugars, and oils. These products tend to possess a natural $^{14}\text{C}/\text{C}$ signature, even if highly processed. It is possible that some components of a processed food are a year or two older, but generally food products are cycled and not stored for multiple years. The tonkatsu sauce and one cake filling in our survey possessed a higher than expected $^{14}\text{C}/\text{C}$ value indicative of being produced around 2002-2004. The diet cola with artificial sweetener possessed an isotope ratio indicative of a mix of carbon from natural and petroleum sources.

The powdered and dried foods in Table 2 possessed a mix of natural and artificial ingredients. Products with $^{14}\text{C}/\text{C}$ values within 5% of the natural range ($F^{14}\text{C} = 0.98$ -1.03) were described as almost natural. Those foods with $^{14}\text{C}/\text{C}$ values more than 5% below the natural range were described as artificial. Both artificial sweetener samples possessed natural $^{14}\text{C}/\text{C}$ signatures. Both were produced by manipulating natural products. All powdered drink mixes in the survey contained artificial sweeteners. Artificial sweeteners did not dominate the ingredients, however, and different flavor formulations produced different isotope signatures. Formulations vary because the different flavors possess different solubilities in water. In some cases emulsifiers are needed to keep

flavorings in the aqueous phase. The flavored gelatin contained sugar as the sweetener but its $^{14}\text{C}/\text{C}$ signature was significantly below natural.

Table 1. Baked goods, cereals, sauces and soft drinks

| <i>Description</i> | <i>F¹⁴C</i> | <i>Assessment</i> |
|-------------------------------|------------------------|-------------------|
| Processed bakery (filling 1) | 1.090±0.004 | Natural |
| Processed bakery (cake 1) | 1.061±0.004 | Natural |
| Processed bakery (frosting 1) | 1.052±0.004 | Natural |
| Processed bakery (filling 2) | 1.048±0.004 | Natural |
| Processed bakery (cake 2) | 1.054±0.004 | Natural |
| Processed bakery (frosting 2) | 1.041±0.004 | Natural |
| Processed bakery (filling 3) | 1.062±0.004 | Natural |
| Processed bakery (cake 3) | 1.055±0.004 | Natural |
| Breakfast cereal | 1.050±0.004 | Natural |
| Cola | 1.054±0.004 | Natural |
| Diet Cola | 0.799±0.004 | Artificial |
| Marshmallow | 1.057±0.004 | Natural |
| Gummy fruit snack | 1.053±0.003 | Natural |
| Hoisin sauce | 1.043±0.004 | Natural |
| Tonkatsu sauce | 1.090±0.004 | Natural |

Table 2. Dried or powdered foods

| <i>Description</i> | <i>F¹⁴C</i> | <i>Assessment</i> |
|---|------------------------|-------------------|
| Non-sugar powdered drink mix (strawberry) | 0.965±0.003 | Artificial |
| Non-sugar powdered drink mix (lemonade 1) | 1.071±0.004 | Natural |
| Non-sugar powdered drink mix (lemonade 2) | 1.023±0.004 | Almost natural |
| Non-sugar powdered drink mix (cherry) | 0.936±0.004 | Artificial |
| Flavored gelatin (powder) | 0.769±0.003 | Artificial |
| Artificial sweetener (powder) | 1.048±0.004 | Natural |
| Artificial sweetener (powder, 2002) | 1.105±0.004 | Natural |
| Gravy mix (powder) | 1.076±0.004 | Natural |
| Ramen spice pack (powder) | 1.047±0.004 | Natural |
| Children's vitamin (dry) | 1.000±0.004 | Almost natural |

The flavorings reported in Table 3 are extracts from specific plants or are clearly identified as imitation. All of these flavorings contained high levels of ethanol which was removed by evaporation. The imitation flavorings surveyed were comprised of predominantly fossil carbon. Unlike extracts that contain hundreds of compounds, the imitation flavorings contain only a couple synthesized compounds to mimick the natural flavoring. The extracts with almost natural $^{14}\text{C}/\text{C}$ ratios were sold as “pure” extracts of lemon, orange and almond. They contained 30-80% ethanol, water, and a small amount of extracted oil of lemon, orange and almond. After evaporation of the ethanol and water, small oil droplets coated the quartz combustion tubes. The small depression from natural $^{14}\text{C}/\text{C}$ was likely due to slight retention of solvents used in the extraction of the oils. The raspberry extract had an unexpectedly low $^{14}\text{C}/\text{C}$ ratio. It was sold as a “real” extract rather than a “pure” extract. It had less ethanol than the other extracts (~20%), but propylene glycol was a significant ingredient. Propylene glycol is generally made from petroleum sources and is a common emulsifier in pharmaceuticals, foods, cosmetics, food colors, and flavorings. The carbon inventory of the raspberry extract was dominated by the propylene glycol rather than the natural components.

Table 3. Flavorings

| <i>Description</i> | <i>F¹⁴C</i> | <i>Assessment</i> |
|--------------------------|------------------------|-------------------|
| Real vanilla | 1.059±0.004 | Natural |
| Imitation vanilla | 0.038±0.001 | Artificial |
| Lemon extract (pure) | 0.983±0.004 | Almost natural |
| Orange extract (pure) | 0.979±0.004 | Almost natural |
| Raspberry extract (real) | 0.114±0.001 | Artificial |
| Almond extract (pure) | 1.000±0.021 | Almost natural |
| Imitation brandy | 0.647±0.002 | Artificial |

The precision of radiocarbon dating depends on the ability to measure the ^{14}C concentration in a sample and characteristics of the calibration curve. The slope of the atmospheric ^{14}C curve varies dramatically over time and the precision of the curve is also variable. It is relatively easy to achieve 0.3-0.5% measurement precision of $^{14}\text{C}/\text{C}$ when analyzing full-sized samples (>300 $\mu\text{g C}$) by AMS. For the given measurement precision, the corresponding chronological range is shorter when the curve is steep (1980) than when it is shallow (2010).

The atmospheric $^{14}\text{C}/\text{C}$ concentration depicted in Figure 1 is not an infinitesimally thin line - it contains uncertainty too. Figure 1 is constructed from published data for clean air collections in a limited number of locations (6-10). Extrapolating the published record for clean air collections to agricultural

production locations introduces uncertainty to the curve. Agricultural products can have local influences which shift the isotopic ratios slightly. These local influences include exposure to polluted air with a slightly depressed $^{14}\text{C}/\text{C}$ ratio (29), variations in soil respiration rates (13), weather conditions which resist flushing of regions with clean air (29), and climatic stresses that effect isotope fractionation (13). Since we seldom have a local atmospheric $^{14}\text{C}/\text{C}$ calibration curve, we need to treat the atmospheric record as a concentration band and include uncertainty in it as well.

Sample size also has an influence on precision. Processing a carbonaceous material into graphite for $^{14}\text{C}/\text{C}$ analysis introduces both contemporary and fossil carbon backgrounds into the sample (26). These background components are established by measuring isotopic standards and ^{14}C -free material over the sample mass range and comparing the measured values to certified values. The contemporary and fossil carbon backgrounds are on the order of $0.8 \pm 0.4 \mu\text{g}$ carbon at LLNL. The uncertainties in these background components have little effect on full-sized samples, but significantly contribute to the uncertainties for samples containing $<40 \mu\text{g}$ carbon. For example, the pure almond extract sample contained only $21 \mu\text{g}$ carbon and its uncertainty is on the order of 2% compared to $\sim 0.4\%$ for the samples containing $\sim 1 \text{ mg}$ carbon.

Conclusions

Analyses of $^{14}\text{C}/\text{C}$ concentrations in foods, flavorings, and health products can be used to determine natural product content. Natural products consist of bio-derived carbon with a $^{14}\text{C}/\text{C}$ concentration of about 1.2 parts per trillion. Artificial ingredients produced from petroleum-derived carbon are devoid of ^{14}C . The recent clean air atmospheric record of $^{14}\text{C}/\text{C}$ in the Northern Hemisphere is data rich for middle and upper latitudes, providing a reliable record of natural product $^{14}\text{C}/\text{C}$ concentrations. All-natural products should contain a $^{14}\text{C}/\text{C}$ signature consistent with the atmospheric record over the past 1-3 years. If a measured $^{14}\text{C}/\text{C}$ concentration is depressed, the product generally contains ingredients synthesized from petroleum-derived carbon.

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